

Alternative Treatments For Melanoma: Targeting BCL-2 Family Members to De-Bulk and Kill Cancer Stem Cells

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For the first time new treatments in melanoma have produced significant responses in advanced diseases, but 30–90% of melanoma patients do not respond or eventually relapse after the initial response to the current treatments. The resistance of these melanomas is likely due to tumor heterogeneity, which may be explained by models such as the stochastic, hierarchical, and phenotype-switching models. This review will discuss the recent advancements in targeting BCL-2 family members for cancer treatments, and how this approach can be applied as an alternative option to combat melanoma, and overcome melanoma relapse or resistance in current treatment regimens.

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THE NEED FOR DEVELOPING ALTERNATIVE MELANOMA TREATMENT OPTIONS

Malignant melanoma is a devastating disease with historically poor prognosis. For the first time, several drugs provide a significant improvement in the overall survival of these patients (Finn *et al.*, 2012; Menaa, 2013; Lo and Fisher, 2014; Tronnier and Mitteldorf, 2014). These drugs fall into two categories: they either block the mitogen-activated protein kinase (MAPK) signaling pathway in BRAF^{V600E}-mutated melanoma, or they alter the patient's own immune system to attack melanoma. Pathway-targeting drugs include BRAF inhibitors that specifically target the BRAF^{V600E} mutation (vemurafenib and dabrafenib) or MEK inhibitors (trametinib and cobimetinib), either alone or in combination (Flaherty *et al.*, 2012). Recent clinical trials with the combinations of both BRAF and MEK inhibitors showed improvements in progression-free survival compared with a single inhibitor in BRAF^{V600E} melanoma patients but also with

increased side effects in some cases (Larkin *et al.*, 2014; Long *et al.*, 2014a,b). Although these treatments lead to a quick initial response, for instance ~50% of patients respond to vemurafenib, responses are not durable (Chapman *et al.*, 2011). Most patients eventually relapse, and the intrinsic or acquired resistance mechanisms include reactivation of MAPK signaling through alternative pathways or activation of other oncogenic pathways such as PI3K/AKT (Fedorenko *et al.*, 2011; Chapman, 2013). In addition, patients without the BRAF^{V600E} mutation do not benefit from these treatments.

For immunotherapy, the humanized monoclonal antibodies ipilimumab and pembrolizumab block the negative regulators of immune responses, thereby stimulating T-cell response against melanoma (Gyorki *et al.*, 2013; Drake *et al.*, 2014). Ipilimumab targets CTLA-4 (Hodi *et al.*, 2010) and pembrolizumab targets PD-1 (Robert *et al.*, 2014). Efficacy with ipilimumab is longer than that with BRAF/MAPK/ERK kinase inhibitors, but the response rate is poor at only 10–15% (Hodi *et al.*, 2010). A distinct genetic landscape of neoantigens is reported to be present in melanomas with a strong response to ipilimumab (Snyder *et al.*, 2014). Moreover, both antibodies lead to significant immune-mediated side effects (Finn *et al.*, 2012), and relapse rate is not yet known for pembrolizumab. Therefore, many patients do not respond to current therapies, and there is still a pressing need to develop alternative treatment options for metastatic melanoma, especially for those with wild-type BRAF.

Human tumors are composed of heterogeneous cell populations. Tumor heterogeneity is implicated in tumor cells' resistance to treatment and may be explained by several models. The stochastic (clonal evolution) model states that every cell within a tumor has similar tumorigenic and self-renewing potential. These cells evolve and lead to heterogeneity, through clonal expansion with acquired genetic and epigenetic changes over time (Nowell, 1976; Shackleton *et al.*, 2009; Greaves and Maley, 2012). This process is influenced by extrinsic factors including therapies. In contrast, the hierarchical model states that only a subpopulation of tumor cells, cancer stem cells (CSCs), possess self-renewal capacity and contribute to intra-tumoral heterogeneity by giving rise to non-CSCs within the same tumor (Reya *et al.*, 2001; Clarke *et al.*, 2006; Frank *et al.*, 2010; Nguyen *et al.*, 2012). This model suggests that eliminating CSCs will help in eradicating tumors. Cancer cell plasticity also contributes to tumor heterogeneity by mechanisms such as phenotype-switching. Cells may switch among proliferative, invasive, and stem cell-like states in response to microenvironments

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Abbreviations: BH, BCL-2 homolog; CSC, cancer stem cell; MAPK, mitogen-activated protein kinase; MSC, melanoma stem cell; SMI, small molecule inhibitor; TIC, tumor-initiating cell

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(Hoek *et al.*, 2008; Hoek and Goding, 2010; Cheli *et al.*, 2011,2012). Moreover, a dynamic process mediated by a temporarily distinct subpopulation may also contribute to melanoma heterogeneity (Roesch *et al.*, 2010). These models/mechanisms are not mutually exclusive. The CSC populations themselves are heterogeneous and dynamic and may evolve through clonal evolution or display phenotype switching (Lee *et al.*, 2014; Nguyen *et al.*, 2015). In melanoma, the high mutation load also likely contributes to heterogeneity. Interestingly, wild-type BRAF/NRAS melanomas seem to have a higher mutation load compared with mutant BRAF/NRAS tumors (Mar *et al.*, 2013).

To prevent relapse, it is proposed that effective therapy needs to eradicate all subpopulations of tumor cells, killing the mass of cancer cells (de-bulking), as well as any resistant subpopulations such as CSCs (Han *et al.*, 2013). One known cancer resistance mechanism is the dysregulation of BCL-2 family members, and recent studies indicate that this also contributes to the survival of CSCs. This review provides an overview of developing new melanoma treatments by targeting BCL-2 dysregulation with the purpose of de-bulking melanomas and eliminating drug-resistant subpopulations of CSCs in melanoma. We include the rationale, methods used and the current promising approaches.

THE REGULATION OF APOPTOSIS BY THE BCL-2 FAMILY

The BCL-2 family of proteins has a crucial role in regulating intrinsic or mitochondrial apoptosis (Czabotar *et al.*, 2014). The members are divided into three groups on the basis of their functions and structures: (i) anti-apoptotic proteins (also called inhibitors), which include Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, A1, and BCL-B; (ii) multi-BH domain pro-apoptotic proteins (also called effectors), which include BAX and BAK; (iii) and BH3-only pro-apoptotic proteins (also called activators or enhancers), which include NOXA, BIM, BID, BAD, BMF, BIK, and others (Figure 1a). Upon activation, the effectors (BAX/BAK) oligomerize, triggering cytochrome C release from the mitochondria and apoptosome activation, and finally result in caspase-9-dependent apoptosis (Czabotar *et al.*, 2014). Anti-apoptotic proteins bind to BAX/BAK, preventing their activation. The balance of the interactions between different BCL-2 family members determines whether cells will initiate apoptosis (Chipuk *et al.*, 2010; Mohana-Kumaran *et al.*, 2014). Some BH3-only members only bind to one group of inhibitors—for example, NOXA only binds to MCL-1/A1. Others can bind to multiple inhibitors, such as BIM, PUMA, and tBID.

BAX/BAK oligomerization is necessary for activation of apoptosis, and the activation has been proposed to be direct or indirect (Czabotar *et al.*, 2014). In the direct activation model, the BH3-only proteins directly bind to effectors (BAX/BAK) leading to activation. In the indirect model, binding of BH3-only proteins to the inhibitors releases the effectors BAX/BAK, allowing for activation. In the indirect model, apoptosis only occurs when all the anti-apoptotic members are neutralized. These two models are not mutually exclusive; one may be more dominant than the other, depending on the

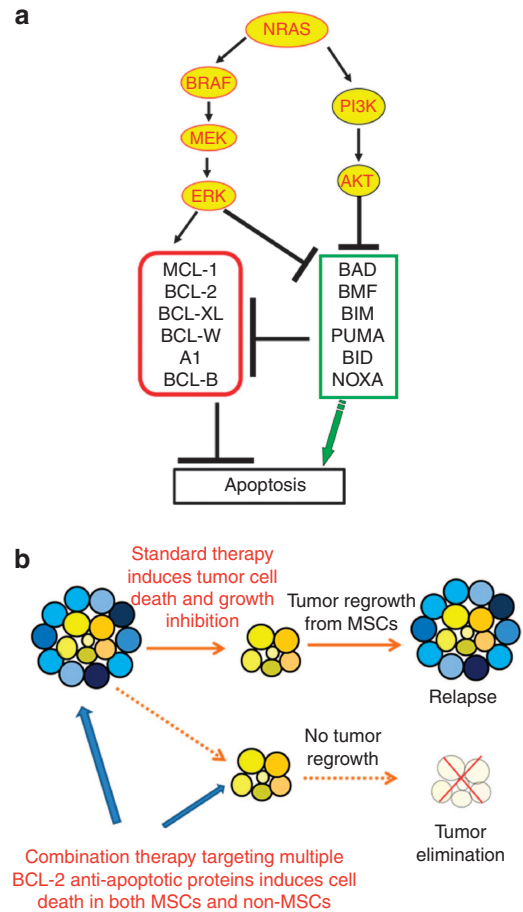


Figure 1. Targeting the BCL-2 family as a treatment option for melanoma.

(a) A simplified illustration for the rationale to target BCL-2 family members in melanoma. Green box represents the pro-apoptotic proteins, and red box represents the anti-apoptotic proteins. Multiple BCL-2 proteins are downstream of the RAS/BRAF/mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling pathways, the commonly activated pathways in melanoma. The activation of these pathways leads to the dysregulated expression of multiple BCL-2 proteins and likely contributes to resistance to cell death in melanoma. For instance, the activated RAS/BRAF/MAPK signal upregulates MCL-1 and blocks BIM and BAD (see text for details). Thus, targeting the BCL-2 family may provide an alternative way to combat melanoma regardless of its BRAF status and to overcome melanoma relapse from current treatments. (b) Combination therapy debulk and kills the melanoma stem cells (MSCs). The various blue cells represent heterogeneous non-MSCs populations, and the yellow cells represent the MSC populations. Standard therapy may be successful in de-bulking melanoma cells initially. However, it fails to kill MSCs, resulting in tumor relapse due to the self-renewal capacity of MSCs. The combination therapy targeting multiple BCL-2 anti-apoptotic members debulks and kills the MSCs, preventing future relapse of melanoma.

circumstances. Nevertheless, in both models, the binding of BH3-only proteins to anti-apoptotic members can activate effectors and induce apoptosis (Czabotar *et al.*, 2014). Thus, mimicking the pro-apoptotic BH3-only proteins by small molecules is a logical approach to develop cancer drugs (Ni Chonghaile and Letai, 2008; Billard, 2013; Mohana-Kumaran *et al.*, 2014).

THE RATIONALE TO TARGET BCL-2 FAMILY MEMBERS IN MELANOMA

Dysregulation of BCL-2 family proteins occurs frequently and has a major role in conferring resistance to cell death in many cancers, including melanoma (Norris, 1995; Plati *et al.*, 2011). Importantly, many BCL-2 proteins are downstream of the commonly activated signaling pathways RAS/BRAF/MAPK and PI3K/AKT (Figure 1a; Kwong and Davies, 2013; Haass and Schumacher, 2014). For instance, oncogenic BRAF^{V600E} suppresses apoptosis by upregulating anti-apoptotic MCL-1 (McKee *et al.*, 2013; Haass and Schumacher, 2014). Also, through phosphorylation, constitutively activated RAS/BRAF/MAPK signals downregulate or inactivate pro-apoptotic BAD (Eisenmann *et al.*, 2003) or BIM (Goldstein *et al.*, 2009). Third, activated PI3K/AKT signals inhibit cell death by inactivating BAD's ability to bind BCL-2 or BCL-XL (Datta *et al.*, 1999; Steelman *et al.*, 2011) or downregulating transcription of pro-apoptotic members BIM and PUMA through sequestering the transcription factors FOXOs away from their promoters (Zhang *et al.*, 2011). Activated AKT also prevents induction of pro-apoptotic BIM-EL and BMF upon BRAF inhibition, contributing to melanoma resistance to BRAF inhibitors (Shao and Aplin, 2010; Figure 1a). Moreover, gene amplification results in upregulation of anti-apoptotic BCL2A1 in some melanoma (Haq *et al.*, 2013). Furthermore, the melanocyte-specific transcription factor (microphthalmia-associated transcription factor) is amplified in a subset of melanoma, which can upregulate BCL-2 and contribute to resistance to cell death (Hartman and Czyz, 2015).

The effects of BRAF inhibitors in melanoma are dependent on the induction of BH3-only protein BIM and BMF (Shao and Aplin, 2010); BIM repression by epigenetic chromatin remodeling (Shao and Aplin, 2012) contributes to melanoma resistance to BRAF inhibition. Considering that some BCL-2 family members are regulated by MAPK or PI3K/AKT signaling and that activation of these pathways contributes to relapses from treatment of BRAF inhibitor, targeting downstream apoptotic proteins may be alternative options to overcome relapse. Indeed, the BAD mimetic, ABT-737, has been shown to overcome AKT-mediated resistance to BRAF inhibition (Perna *et al.*, 2015).

Taken together, multiple mechanisms lead to dysregulation of the BCL-2 family, and this likely contributes to melanoma development of resistance to current treatments (Figure 1a). Thus, targeting the BCL-2 family is an alternative way to combat melanoma, independent of BRAF mutation status, and overcome melanoma relapse from current treatments.

DE-BULKING CANCER CELLS BY TARGETING BCL-2 FAMILY MEMBERS

Single agents targeting BCL-2 or MCL-1

Early attempts at targeting BCL-2 family members included antisense, single-chain antibodies, ribozymes, BH3 peptides, and hydrocarbon stapling. However, most failed, possibly because of poor delivery systems and the short-term stability of the compounds (see review Thomas *et al.*, 2013). Current more successful approaches focus on stable small molecule inhibitors (SMI), called "BH3 mimetics", which mimic the

pro-apoptotic BH3-only proteins to induce apoptosis in cancer cells (Table 1).

Currently, ABT-263 (navitoclax) and ABT-199 are in clinical trials, with encouraging preliminary results (Wilson *et al.*, 2010; Gandhi *et al.*, 2011; Roberts *et al.*, 2012). ABT-263, an oral version of ABT-737, is a mimetic of BH3-only BAD that inhibits BCL-2, BCL-XL, and BCL-W but not MCL-1 (Oltersdorf *et al.*, 2005; van Delft *et al.*, 2006; Tse *et al.*, 2008). ABT-199 was modified from ABT-263, with a sub-nano-molar affinity for BCL-2 but not BCL-XL (Davids and Letai, 2013). Unfortunately, single agent treatment of melanoma with ABT-737/ABT-263 is not effective, with MCL-1 as the main contributor of resistance (Chen *et al.*, 2007; Miller *et al.*, 2009; Lucas *et al.*, 2012).

MCL-1 overexpression is linked to the pathogenesis of multiple cancers, including melanoma (Boisvert-Adamo *et al.*, 2009; Khodadoust *et al.*, 2009; Thomas *et al.*, 2010; McKee *et al.*, 2013). Many groups are developing SMIs that target MCL-1 with the aim of overcoming cancer resistance to ABT-737/263. Promising compounds include Maritoclax, WP1130, UMI-77, Clitocine, and Compound 11, with some efficacy in animal studies (Quinn *et al.*, 2011; Wei *et al.*, 2012; Belmar and Fesik, 2014; Sun *et al.*, 2014). However, clinical trials are still needed (Table 1).

Combination therapy

As mentioned above, targeting a single BCL-2 family member is not sufficient to kill melanoma. In addition, therapeutics with single molecular targets often fails because of the heterogeneity and dynamic nature of cancer cells. Thus, utilizing combination therapies is an emerging strategy to treat cancer. Combination therapy increases the number of cells responding to treatment, decreases the possibility of drug resistance, kills heterogeneous populations of cells within the tumor, and may also lower side effects.

Several combination strategies target BCL-2 family members, with promising pre-clinical evidence (Table 1). As mentioned earlier, MCL-1 is the main mediator of melanoma resistance to BCL-2 inhibitors such as ABT-737, and NOXA is a pro-apoptotic BH3-only protein that inhibits MCL-1. Therefore, inhibiting BCL-2, combined with either inhibition of MCL-1 or activation of NOXA, is effective at killing melanoma (Miller *et al.*, 2009; Reuland *et al.*, 2011; Lucas *et al.*, 2012; Reuland *et al.*, 2012; Mukherjee *et al.*, 2015). Another strategy is sensitizing melanoma with BCL-2 SMIs, coupling with molecular-targeted agents such as BRAF inhibitors (Wroblewski *et al.*, 2013). The last strategy combines BCL-2 SMIs with immune therapies, again using BH3 mimetics such as ABT-737, to sensitize tumor cells to immunotherapies (Begley *et al.*, 2009; Karlsson *et al.*, 2013). All these approaches provide treatment opportunities that may limit escape mechanisms in different patients and enhance the possibilities for personalized cancer medicine approach to treatment. Although the above strategies are promising, none explored the effectiveness of specifically eradicating resistant subpopulations, which is an important contributor for melanoma resistance.

Table 1. Examples of SMIs targeted BCL-2 family members in clinical trials or pre-clinical studies

SMI	Target(s)	Clinical trials or pre-clinical studies		
		Cancer type	Combination drug	Clinical trials ^a
Gossypol	BCL-2	Adrenocortical carcinoma	NA	II
	BCL-XL	Lymphoma	Paclitaxel, carboplatin	I
	MCL-1	Advanced SCLC, solid tumors	Cisplatin, etoposide	I
	BCL-W	Advanced prostate cancer	Bicalutamide	II
	BCL-B			
Obatocicax	BCL-2	Advanced SCLC	Carboplatin, etoposide	I/II
	MCL-1	AML	NA	II
	BCL-XL	CLL	NA	I/II
	BCL-W	Hodgkins lymphoma	NA	II
	BCL-B	MCL, Lymphoma	Bortezomib	I/II
	BFL-1	NSCLC	Docetaxel	I/II
ABT-263 (ABT-737)	BCL-2	SCLC	Topotecan hydrochloride	I/II
	BCL-XL	CLL, SCLC, Leukemia	NA	II,I,II
	BCL-W	Lymphoma, CLL, solid tumors	Ketoconazole	I
		CLL	Fludarabine, Cyclophosphamide, Rituximab	I
		Solid tumors	Doxetaxel, Gemcitabine, Etoposide, Cisplatin, Paclitaxel	I
ABT-199		Lymphoma; CLL	Rifampin	I; II
Maritocicax	BCL-2	SLL, NHL, CLL, AML	NA	I; II
		B-cell lymphoma	Rituximab	IB
Clitocine	MCL-1	Melanoma	Single and with ABT-737; <i>in vitro</i> and <i>in vivo</i>	NA
		AML	Single; <i>in vitro</i>	
UMI-77	MCL-1	Hepatocellular carcinoma	Single; <i>in vitro</i> and <i>in vivo</i>	NA
UMI-77	MCL-1	Pancreatic cancer	Single; <i>in vitro</i> and <i>in vivo</i>	NA

Abbreviations: AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; NA, not available; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; SLL, small lymphocytic leukemia; SMI, small molecule inhibitor.

None of the MCL-1 inhibitors listed are in clinical trials yet; thus, we provided the information based on the pre-clinical studies for these instead. More details can be found in the reviews by Belmar and Fesik, 2014; and Thomas *et al.*, 2013.

^aThe information for clinical trials is adapted from www.clinicaltrials.gov

CSCs IN MELANOMA

CSCs are defined as a subpopulation of cancer cells that possess two essential stem cell functions: self-renewal and differentiation (Clarke *et al.*, 2006). CSCs enhance cancer initiation, progression, metastasis, and chemoresistance (Clarke *et al.*, 2006; Nguyen *et al.*, 2012; Pattabiraman and Weinberg, 2014). CSCs also contribute to intra-tumoral heterogeneity, with their ability to give rise to non-CSCs within the same tumor (Frank *et al.*, 2010; Pattabiraman and Weinberg, 2014). To prevent relapse, targeting CSCs should be an important part of treatment strategies.

The gold standard method to confirm the existence of CSCs is to detect their capacity to self-renew and differentiate, using *in vivo* serial xenotransplantation assays (Clarke *et al.*, 2006). Recently, several lineage tracing studies with genetically engineered mouse models provided further evidence for the existence of CSCs (Pattabiraman and Weinberg, 2014). To date, CSCs have been identified in many cancers (Tabarestani and Ghafouri-Fard, 2012; Pattabiraman and Weinberg, 2014).

Quintana and colleagues reported a high frequency of tumor-initiating cells (TICs) in human melanoma using highly permissive conditions, raising questions on the existence of CSCs in human melanoma (Quintana *et al.*, 2008). However, TICs are not the same as CSCs with self-renewal and differentiation capacity (Shakhova and Sommer, 2013), and

the high frequency of TICs does not disprove the presence of CSCs (see reviews (Lang *et al.*, 2013; Shakhova and Sommer, 2013; Lee *et al.*, 2014; Nguyen *et al.*, 2015)). Rather, Quintana's papers simply suggest that TICs are influenced by many experimental factors. Indeed, when using the gold standard *in vivo* assays, numerous groups have detected subpopulations of melanoma cells that fulfill the criteria for CSCs (Lang *et al.*, 2013; Shakhova and Sommer, 2013; Lee *et al.*, 2014; Nguyen *et al.*, 2015), and these cells are associated with tumor progression and chemoresistance.

Several melanoma stem cell (MSC) markers have been suggested in peer-reviewed literatures, and these include cell surface markers ABCB5 (Schatton *et al.*, 2008) and CD271 (Boiko *et al.*, 2010; Civenni *et al.*, 2011; Redmer *et al.*, 2014) and activity of aldehyde dehydrogenase (a detoxifying enzyme; Luo *et al.*, 2012). ABCB5 belongs to the ATP-binding cassette transporter superfamily, implicated in multi-drug resistance. CD271 is a nerve growth factor receptor and is also a marker of mesenchymal stem cells (Watson *et al.*, 2013) and hypopharyngeal CSCs (Imai *et al.*, 2013). However, recent evidence suggests that not all CD271⁺ cells are the same, and only the slow growing CD271⁺ population displays a high tumorigenic potential and stemness (Cheli *et al.*, 2014). In addition, knockdown of CD271 alone decreases, but does not prevent, the accumulation of the

stress-induced drug-resistant cell population (Menon *et al.*, 2015). These recent data suggest that CD271 may be an imperfect MSC marker.

In sum, subpopulations of melanoma cells possess characteristics of CSCs (tumorigenesis with self-renewal and differentiation capacities), with enhanced ability for melanoma initiation, maintenance, and resistance to treatments. Although various markers can enrich MSCs, the most critical functional trait of these subpopulations is their self-renewal capacity for long-term growth of melanoma. Therefore, self-renewability of these subpopulations should be targeted to prevent relapse after treatment.

ERADICATING CSCs BY TARGETING BCL-2 FAMILY MEMBERS

Ideally, cancer treatments should eliminate both non-CSCs (the bulk of the tumors) and CSCs (Frank *et al.*, 2010; Rameshwar, 2014). Utilizing combination therapy is likely the best strategy for targeting all subpopulations in heterogeneous tumors, and promising new drugs should test the efficacy of killing CSCs.

Potential strategies that focus on CSCs include differentiating CSCs into mature cells with no self-renewal capacity, eliminating CSCs by targeting essential pathways, or disrupting their niche. A successful example of differentiation therapy is the use of all-trans retinoic acid on acute promyelocytic leukemia patients to differentiate the cancer cells into mature granulocytes (Pattabiraman and Weinberg, 2014). Another approach to kill CSCs is through targeting their dependent signaling pathways, such as NOTCH, ATP-binding cassettes, NF- κ B, transforming growth factor- β , WNT, and JAK-signal transducer and activator of transcription (Chen *et al.*, 2013; Pattabiraman and Weinberg, 2014). In addition, tumor microenvironment may serve as CSC's niche and can also be a drug target to eliminate CSCs. For example, angiogenesis is crucial in maintaining tumor niches, and the melanoma niche encompasses a network consisting of not only endothelial-lined blood vessels but also "vasculogenic mimicry" channels formed by melanoma cells (Lai *et al.*, 2012). Drugs targeting angiogenesis (such as vascular endothelial growth factor inhibitors) can disrupt tumor niche and indirectly eradicate CSCs (Schatton *et al.*, 2008; Frank *et al.*, 2011; Lee *et al.*, 2014; Pattabiraman and Weinberg, 2014).

Numerous recent studies show that CSCs are especially vulnerable to SMIs targeting the BCL-2 family. Lagadinou *et al.* (2013) showed that BCL-2 was overexpressed in quiescent leukemia stem cells with low levels of reactive oxygen species, and BCL-2 inhibitor ABT-263 selectively eradicated the leukemia stem cells (Lagadinou *et al.*, 2013). In bone-marrow-resident human leukemia stem cells, splice-isoform switching favored expression of multiple pro-survival BCL-2 members, and a pan-BCL-2 inhibitor sensitized them to tyrosine kinase inhibitors (Goff *et al.*, 2013). Other CSCs, such as breast and colon CSCs, displayed upregulated BCL-2 (Fulda, 2013). BCL-XL overexpression contributed to survival of colon CSCs (Colak *et al.*, 2014) and lung CSCs (Zeuner *et al.*, 2014). When treated with ABT-737 but not ABT-199, these cells died (Zeuner *et al.*, 2014) or were sensitized to

chemotherapies (Colak *et al.*, 2014). Furthermore, suppressing MCL-1 may overcome the tumor-plasticity-induced drug resistance in multiple myeloma cells (Dalva-Aydemir *et al.*, 2014). These findings highlight that inhibition of pro-survival BCL-2 family proteins is a promising approach to target chemotherapy-resistant CSCs and plasticity of tumors.

To our knowledge, few studies explored the BCL-2 family members as targets for eradicating heterogeneous melanomas or MSCs. Recently, we identified the combination of a BCL-2 Inhibitor with the retinoid derivative fenretinide as a promising treatment, for both wild-type and mutant BRAF cells and for non-MSCs and MSCs (Mukherjee *et al.*, 2015; Figure 1b). The combination synergistically inhibited melanoma growth *in vitro* and *in vivo*, disrupted melanoma spheres, decreased the percentage of ALDH^{high} cells, and inhibited the self-renewal capacity of MSCs. These effects were observed in melanoma cells with mutations of either BRAF or NRAS. Interestingly, single drug treatments increased characteristics of MSCs for some melanoma samples, and only the combination treatment significantly reduced the self-renewal capacity of MSCs in all the samples tested. Proliferation stopped post treatment, with no re-growth of tumor cells. The mechanism of action for the combination involves antagonizing multiple anti-apoptotic BCL-2 members at once (Mukherjee *et al.*, 2015; Figure 1b). These results support the idea that combination treatments are more potent to eliminate MSCs or other resistant subpopulation and targeting multiple pro-survival BCL-2 family members is a promising approach for melanoma (Figure 1b).

SUMMARY

By killing heterogeneous tumors and eliminating drug-resistant subpopulations, SMIs, targeting multiple BCL-2 family members, provide an option for melanoma, especially the wild-type BRAF melanomas. This approach thus offers an alternative way to combat melanoma and may help achieve longer lasting treatment effects.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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